against a number of both Gram positive and Gram negative bacteria. Its lethal dose for albino rats has been found to be more than 250 mg per kg body weight.

The author wishes to express his deep gratitude to Professor Samuel H. Zia for his constant advice and helpful criticisms during this investigation.

16645

Anticoagulant Effect of Dicumarol at Various Prothrombin Levels in Dogs.

N. HENRY MOSS, RICHARD L. SCHAFER, AND CHARLES K. KIRBY.
(Introduced by I. S. Ravdin.)

From the Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia.

Since the introduction of dicumarol for clinical use[1] there have been differences of opinion concerning the prothrombin reduction required to produce an adequate anticoagulant effect. Some investigators recommend that the prothrombin level be reduced to 10 to 30% of normal.[2,3,4] Others state that the effective therapeutic prothrombin zone lies between 20 and 60%;[5] 35 and 50%[6] and 50 and 60%.[7]

In 2 reported series of patients in which the prothrombin was reduced to 10 to 30% of normal, hemorrhage occurred in 5%[3] and 6%[4] of the patients. Excessive hypoprothrombinemia (below 10%) occurred in 10 to 20% of patients following doses of dicumarol which usually resulted in 10 to 30% prothrombin levels.[4] It seems probable, therefore, that the maintenance of higher prothrombin levels might be preferable if the anticoagulant effect is adequate at higher levels.

Little experimental work has been done to clarify this problem. In the experiments herein reported the anticoagulant effect of dicumarol at various prothrombin levels in dogs has been studied in order to determine whether there is a definite and consistent correlation between prothrombin levels and anticoagulant activity.

Method. The dogs were anesthetized with intravenous sodium pentobarbital, and the femoral artery was exposed and cannulated with as little trauma as possible. Thrombosis was produced by inserting into the femoral artery a sterile glass cannula 1.0 mm in diameter at its constricted opening. The cannula widened out to a 3.5 mm diameter where it was connected to a mercury manometer by sterile rubber tubing. The entire system was filled with sterile physiological saline solution. Additional saline was introduced through a T side-arm in the tubing to produce a positive pressure approximately equal to the mean arterial blood pressure in order to prevent the passage of blood into the tubing. When thrombosis occurred, the mercury column in the manometer ceased oscillating, and this was taken as the end-point.

The prothrombin level of each dog was determined within two hours of the time it was cannulated by the Quick method, with the plasma diluted 50% with normal physiological saline solution. A normal curve was plotted each day the experiment was performed to eliminate errors due to a variation in the potency of the thromboplastin. Table I expresses the prothrombin times (in seconds) of various percentages of normal plasma (50%

1 Allen, E. V., Barker, N. W., and Waugh, J. M., *J.A.M.A.*, 1942, **120**, 1009.

2 Barker, N. W., *Minnesota Med.*, 1944, **27**, 102.

3 Allen, E. V., *J.A.M.A.*, 1947, **134**, 323.

4 Cosgriff, S. W., Cross, R. J., and Habif, D. V., *Surg. Clin. N. Am.*, April 1948, 324.

5 Levan, J. B., *Ann. Int. Med.*, 1946, **25**, 941.

6 Peters, H. R., Guyther, J. R., and Brambel, C. E., *J.A.M.A.*, 1946, **130**, 398.

7 Cummine, H., *M. J. Australia*, 1947, **34**, 302.

TABLE I.
Plasma Prothrombin Times (in Seconds) of Normal Healthy Dogs.

Percentage dilution of normal control plasma (50% diluted)	Total No. of determinations	Avg time (sec)	Range of times (sec)
100	95	9.9	8.2-11.5
90	18	10.2	9.0-11.5
80	21	10.3	9.0-11.8
75	35	10.8	9.4-12.9
60	31	11.0	9.6-13.9
50	55	11.3	9.8-14.8
40	39	12.4	10.0-17.6
30	43	14.0	10.5-19.8
25	36	14.7	12.7-21.6
20	51	17.1	12.3-26.1
15	33	18.7	15.3-32.3
10	47	27.6	18.2-50.7
5	8	38.0	35.9-41.0

diluted) and shows the averages and ranges obtained.

Results. In a control series of 14 normal animals the femoral arteries were cannulated 22 times. Thrombosis occurred, on the average, in 6.3 minutes, with a variation from 3 to 10 minutes (Fig. 1).

The experimental series consisted of 32 dogs ranging in weight from 8.0 kg to 14.2 kg. Dicumarol in doses from 10.0 mg to 400 mg was administered orally 72 hr before the experimental procedure. Prothrombin levels ranging from 95% to 8% of normal were obtained following this therapy.

Prolongation of the "thrombosis time" was found to occur at prothrombin levels of 40% and below (Fig. 2). Of 23 dogs with levels below 40%, the average time was 16.6 minutes. In 20 dogs the thrombosis time was prolonged beyond the longest time of any in the control series. In the remaining 3 thrombosis occurred (10, 10 and 9 minutes) at about the same time as the longest in the control series (10 minutes). The average prolongation of the thrombosis time did not vary significantly at 10, 20, 30 and 40% prothrombin levels.

Prolongation of the "thrombosis time" was not found to occur in dogs with prothrombin levels of 44% or higher. All of the nine animals in this group had "thrombosis times" within the limits of the controls, the average being 7.2 minutes.

In 10 of the dicumarolized animals with prothrombin levels from 8 to 95%, there was no correlation between the Lee-White coagulation time and the cannula "thrombosis time".

Comment. Advantages of the method used are that: (1) the endpoint is clear cut; (2) thrombosis occurs in a relatively short period of time; and (3) the time of occurrence of thrombosis in control animals is relatively constant. Since thrombosis occurred in or about the cannula, this is not strictly an *in vivo* method of producing throm-

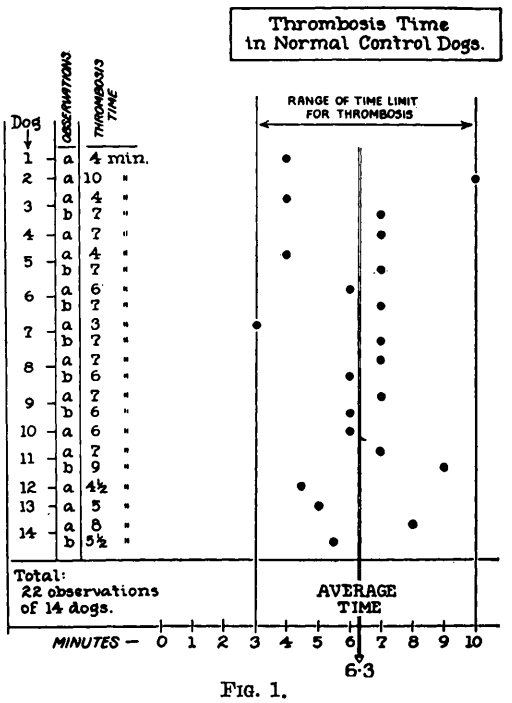

FIG. 1.

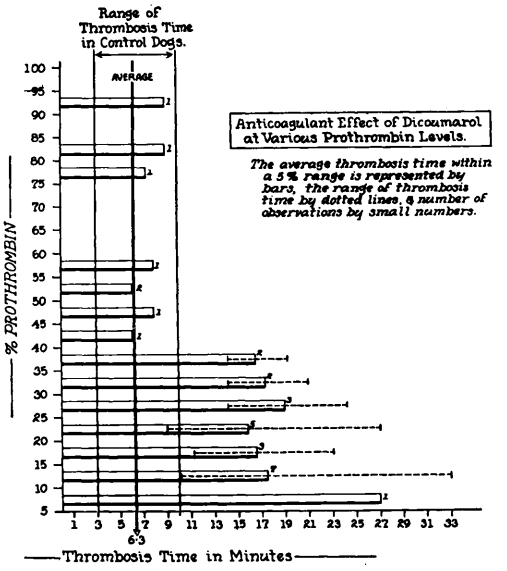

FIG. 2.

bosis, as compared with clinical thrombosis. Neither is it an *in vitro* method, as shown by the lack of correlation between the Lee-White coagulation time and the cannula "thrombosis time". For the purpose sought in this experiment of obtaining a comparative quantitative estimate of the anticoagulant activity of dicumarol at various prothrombin levels, the method has appeared adequate. The method of producing thrombosis used in this experiment obviously does not simulate clinical conditions in which thrombosis occurs. A more suitable method of producing experimental thrombosis has not been devised so far as we know.

The two striking results in the experiment are: (1) the sharp line of demarcation of anticoagulant activity at a prothrombin level of 40% and (2) the apparent uniformity of the average anticoagulant activity at 10, 20, 30 and 40% prothrombin levels.

It is to be emphasized that under the conditions of these experiments the response to dicumarol in dogs may bear little relationship to dicumarol response in man. Yet it is apparent that in dogs: (1) prothrombin levels above 40% had no demonstrable effect upon prolongation of the thrombosis time and (2) prothrombin levels below 40% did significantly increase the thrombosis time in 20 of 23 experiments.

In view of these results and the lack of conclusive experimental evidence elsewhere, the question must be raised as to whether the clinical use of prothrombin levels of 10% to 30% may be too low or whether levels of 40% to 60% may be too high for effective yet safe anticoagulant therapy.

As to the apparent uniformity of anticoagulant activity at 10, 20, 30 and 40%, this may merely reflect a limitation of the sensitivity of the method or else may reveal that an all or none, rather than a mass action phenomenon, is associated with the method. This cannot be definitely answered at present from the data available.

Conclusions. 1. The anticoagulant effect of dicumarol at various prothrombin levels in dogs has been studied.

2. In control animals thrombosis occurred, on the average, in 6.3 minutes, with a variation from 3 to 10 minutes.

3. In dicumarolized dogs the time of thrombosis was not delayed at prothrombin levels above 40%. Below 40% thrombosis occurred on the average, in 16.6 minutes.